

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



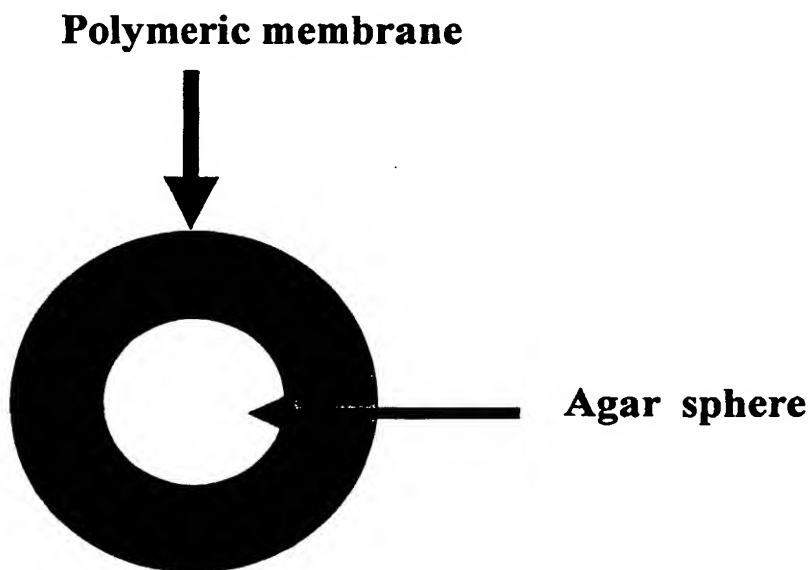
(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/022698 A2

- (51) International Patent Classification⁷: C12N (74) Agent: BEN-AMI, Paulina; Ben-Ami & Associates, P. O. BOX 94, 76100 Rehovot (IL).
- (21) International Application Number: PCT/IL2003/000725 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 3 September 2003 (03.09.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 151660 9 September 2002 (09.09.2002) IL (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): BEN GU-RION UNIVERSITY OF THE NEGEV RESEARCH AND DEVELOPMENT AUTHORITY [IL/IL]; P.O. Box 653, 84105 Beer-Sheva (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): KUSHMARO, Ariel [IL/IL]; 19 Dekel St., 72905 Moshav Nir-Zvi (IL). GERESH, Shimona [IL/IL]; 27 Tapuz St., 84965 Omer (IL).
- Published:
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: METHOD FOR ISOLATING AND CULTURING UNCULTURABLE MICROORGANISMS



(57) Abstract: The invention provides a method for isolating and culturing a previously unculturable microorganism, which comprises: (i) collecting a sample from an environmental source; (ii) counting/estimating the number of microorganisms in the sample; (iii) diluting the sample in an appropriate medium; (iv) adding a gelating agent such as to entrap one or more microorganisms within a sphere of the gelating agent; (v) coating the spheres containing the entrapped microorganism(s) with a natural or synthetic polymer to form a polymeric membrane; (vi) incubating the coated spheres in the original environment for an appropriate time; (vii) cutting the spheres and scanning for microorganisms colonies; and (viii) isolating the microorganisms, and repeating steps (iii) to (vii) until a pure clone of said previously unculturable microorganism is obtained.

BEST AVAILABLE COPY

WO 2004/022698 A2